Docket No.: VASG-P02-003
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Gill et al

Application No.: 09/487023

Group Art Unit: 1635

Filed: January 19, 2000

Examiner: McGarry, S.

For: METHOD AND COMPOSITION FOR TREATMENT OF KAPOSI'S SARCOMA

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 35 U.S.C. §1.132 OF PARKASH GILL

DR. PARKASH GILL hereby declares and states as follows:

1. I am a named inventor of the above-identified patent application, and the subject matter described and claimed therein.

2. I am Professor of Medicine and Pathology at the Keck School of Medicine at the University of Southern California. I have worked on the development of clinically useful antisense nucleic acids for over 10 years. A copy of my CV is enclosed with this Declaration.

3. **The Office Action:** I have read the Office Action issued by the U.S. Patent and Trademark Office on November 19, 2003 in the above-identified patent application. I have also reviewed the references cited in the Office Action, including Uchida et al., U.S. Patent No. 6,150,092. I understand that the Examiner has rejected the pending claims as obvious in view of Uchida et al. in combination with various other references. From a scientific perspective, I do not believe that the Uchida et al. reference renders the claims of the present application obvious.

Had I read the Uchida et al. reference at the time that the present application was filed, January 19, 2000, I would not have been motivated to generate phosphorothioate (PS) modified forms of the VEGF antisense nucleic acids set forth in Uchida et al. I base this conclusion on reasons set forth below.

4. **Phosphorothioate-modified antisense probes:** First, I note that PS-modified antisense probes are designed for use in *in vivo* or cell-based applications of antisense technology. This is because PS-modified nucleic acids have improved resistance to nucleases found in cells. One would have no reason to make a PS-modified form of an antisense nucleic acid unless one intended to use the antisense construct to affect gene expression in cells. Therefore, a demonstration that an unmodified antisense probe is effective in a cell-free assay would not necessarily provoke one to make and test the PS-modified form in a cell-based assay. This is particularly true where, as with Uchida et al., there is a poor correlation between the results seen in the cell-free and cell-based assays.

5. **Cell-free Assays:** Uchida et al. describe experiments testing antisense probes for their effects on VEGF expression. The majority of the data presented by Uchida et al. were based on cell-free assays employing unmodified DNA antisense probes at a concentration of 0.4 micromolar (see, Tables 1-8 of Uchida et al. and col. 20, line 3). Tables 1 and 2 in particular show that dozens of unmodified antisense probes were effective in decreasing VEGF expression in the cell-free assay. In many instances VEGF expression was decreased by greater than 90%.

6. **Cell-based Assays:** Uchida et al. selected six probes that were highly effective in the cell-free assays and tested PS-modified forms of these probes in a cell-based assay. The effects of these probes on VEGF expression in cells are shown in Table 9. The amount of VEGF expression observed by Uchida et al. in the presence of the PS-modified probes was high, ranging from 54% to 70% of normal (59% to 82% when corrected for the baseline inhibition seen in the controls).

The cell-based assays of Uchida et al. were performed with PS-modified probes at the high concentration of 20 micromolar (Col. 25, line 30). The cell-free assays in Tables 1 and 2 were performed at a concentration of 0.4 micromolar. At a concentration of 20 micromolar, it is

often difficult to discern whether an effect on gene expression is due to a specific antisense effect or a generalized effect on cellular processes. Given the high probe concentration and the small observed effect on VEGF expression in the cell-based assays, I conclude that the antisense probes identified by Uchida et al. are not effective for inhibiting VEGF expression in cells. These data would not have motivated me to modify and test other probes disclosed by Uchida et al.
for use in cells.

7. **A Comparison of the Cell-free and Cell-based Assays:** There is a poor correlation between the effectiveness of the unmodified probes in the cell-free assays and the PS-modified probes in the cell-based assays. For example, the unmodified probe A311 (SEQ ID NO:51) inhibited 96% of VEGF expression in the cell-free assay, but the PS-modified form of A311 inhibited only 22% to 28% of VEGF expression in the cell-based assay (at a 50-fold higher concentration). Of six probes that were effective in cell-free assays, all six showed only mild effect on VEGF expression in the cell-based assay. I conclude that there is no reason to expect that any of the probes that Uchida et al. identified in the cell-free assay would be likely to be effective as PS-modified forms.

8. I further state that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: July 30 - 2005 Signed: _____

Dr. Parkash Gill

ABBREVIATED CURRICULUM VITAE

NAME: Parkash S. Gill, M.D.

CURRENT POSITION: Professor of Medicine (Hematology, Oncology and Pathology)
University of Southern California Keck School of Medicine

BOARD CERTIFICATION: American Board of Internal Medicine, 1982
Medical Oncology, 1987

PROFESSIONAL TRAINING:

1974	M.D. Medicine Government Medical College
1977-1981	Resident, New Brunswick Affiliated Hospitals, N.J.
1981-1983	Fellowship, LAC & USC Medical Center -Hematology

PROFESSIONAL & SCIENTIFIC GROUP MEMBERSHIPS:
American Society of Hematology
American Society of Clinical Oncology
American Association for the Advancement of Science

SELECTED PUBLICATIONS IN THE ANTISENSE FIELD:

1: Xia G, Kumar SR, Masood R, Koss M, Templeman C, Quinn D, Zhu S, Reddy R, Krasnoperov V, Gill PS. Up-regulation of EphB4 in mesothelioma and its biological significance.Clin Cancer Res. 2005 Jun 15;11(12):4305-15.

2: Xia G, Kumar SR, Masood R, Zhu S, Reddy R, Krasnoperov V, Quinn DI, Henshall SM, Sutherland RL, Pinski JK, Daneshmand S, Buscarini M, Stein JP, Zhong C, Broek D, Roy-Burman P, Gill PS. EphB4 expression and biological significance in prostate cancer. Cancer Res. 2005 Jun 1;65(11):4623-32.

3: Hotz HG, Hines OJ, Masood R, Hotz B, Foitzik T, Buhr HJ, Gill PS, Reber HA. VEGF antisense therapy inhibits tumor growth and improves survival in experimental pancreatic cancer. Surgery. 2005 Feb;137(2):192-9.

4: Masood R, Kundra A, Zhu S, Xia G, Scalia P, Smith DL, Gill PS.
Malignant mesothelioma growth inhibition by agents that target the VEGF and VEGF-C autocrine loops. Int J Cancer. 2003 May 1;104(5):603-10.

5: Masood R, Cesarman E, Smith DL, Gill PS, Flore O.
Human herpesvirus-8-transformed endothelial cells have functionally activated vascular endothelial growth factor/vascular endothelial growth factor receptor. Am J Pathol. 2002 Jan;160(1):23-9.

6: Masood R, Cai J, Tulpule A, Zheng T, Hamilton A, Sharma S, Espina BM, Smith DL, Gill PS. Interleukin 8 is an autocrine growth factor and a surrogate marker for Kaposi's sarcoma. Clin Cancer Res. 2001 Sep;7(9):2693-702.

7: Masood R, Cai J, Zheng T, Smith DL, Hinton DR, Gill PS.
Vascular endothelial growth factor (VEGF) is an autocrine growth factor for VEGF receptor-positive human tumors. Blood. 2001 Sep 15;98(6):1904-13.

8: Masood R, Cai J, Zheng T, Smith DL, Naidu Y, Gill PS.
Vascular endothelial growth factor/vascular permeability factor is an autocrine growth factor for AIDS-Kaposi sarcoma. Proc Natl Acad Sci U S A. 1997 Feb 4;94(3):979-84.

9: Mori N, Gill PS, Mougdil T, Murakami S, Eto S, Prager D. Interleukin-10 gene expression in adult T-cell leukemia. Blood. 1996 Aug 1;88(3):1035-45.

10: Cai J, Gill PS, Masood R, Chandrasoma P, Jung B, Law RE, Radka SF. Oncostatin-M is an autocrine growth factor in Kaposi's sarcoma. Am J Pathol. 1994 Jul;145(1):74-9.